1,074,235

PATENT SPECIFICATION

DRAWINGS ATTACHED

1,074,235

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COMPLETE SPECIFICATION

Antibiotics R-451B(x) and R-451D(x) and Processes for their Manufacture

We, SCHERICO LTD., of Winkelriedstrasse 56, Lucerne, Switzerland, a body corporate constituted under the laws of Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new antibiotics, to a process for their production from precursor antibiotics, and to therapeutic compositions containing them.

In the specification of our British patent No. 1,021,402 there is described *inter alia* an antibiotic designated therein as R-451, which is characterized, and identified, by certain physical parameters including melting point, solubility in different solvents, elementary analysis, rotational and spectral (infrared and ultra-violet) data and anti-bacterial activity. This antibiotic is produced by a fermentation process which is also described in the aforementioned specification. In its preferred form this fermentation process involves the incubation, in an aqueous nutrient medium providing assimilable carbon and nitrogen, under submerged aerobic conditions, of a micro-organism belonging to the species *Micromonospora carbonacea*. Typically, the fermentation process is carried out at a pH in the range of 6 to 8, over a period of about 60 to 72 hours, which is adequate to impart substantial antibiotic activity to the culture medium. The antibiotic R-451 is then isolated from the medium. This isolation generally involves, as a first step, the separation of the broth of the culture medium from the mycelium, and then extracting the antibiotic by adsorption, partition or fractional precipitation.

Considerable information concerning various features of the antibiotic No. 451 and the fermentation process for producing it is given in the aforementioned specification but, of particular important, in the context of this invention, is the description therein of the actual constitution of the antibiotic R-451 which, as evidenced by chromatographic studies, is in the form of a mixture of at least five components, referred to as R-451A, R-451B, R-451C, R-451D and R-451E respectively, although the number of components which can be resolved in a particular instance depends upon the method employed. The various components, all of which manifest antibiotic activity, are present therein in different proportions although component R-451D often constitutes a major proportion of the mixture. The resolution of R-451 into its components is typically effected by partition chromatography, although other methods known (in the sense of being in actual use or described in the literature) for the resolution of a fermentation product into components may be employed. The components following upon their isolation may be transformed into a derivative, the derivatives mentioned being ether derivatives (for instance, methyl ethers), O-acyl derivatives, such, for example, as acetates, and salts with bases.

This invention, which is an improvement in or modification of the invention described in the aforementioned specification, provides two new antibiotics derived from two of the components of the antibiotic R-451, namely R-451B and R-451D, herein sometimes for convenience referred to as precursor antibiotics. These new antibiotics have characteristics which, in certain respects at any rate, are difference from those of the precursor antibiotic. In view of these differences, the new antibiotics are, in the strict sense, new substances and accordingly, they shall herein be distinguished from the precursor antibiotics, R-451B and R-451D, by the designation R-451B(X) and R-451D(X).

[*Price 4s. 6d.*]

Among the more significant differences in the characteristics of the antibiotics R-451B(X) and R-451D(X) on the one hand, and the precursor antibiotics R-451B and R-451D on the other, are melting points, with the new antibiotics having higher melting points than the precursor antibiotics, and spectral data including infra-red and ultra-violet absorption spectra.

Further, the new antibiotics have superior biological properties such as an enhanced activity, as evidenced by the assay value, against many gram positive organisms. Thus, R-451B(X) has an assay value of 760 units/mg compared with a value of 700 units/mg for R-451B, while R-451D(X) has an assay value of 1450 units/mg compared with a value of 1155 for R-451D. The increased assay values indicate that the new antibiotics are more potent, having lower minimum inhibitory concentrations, than the precursor antibiotics.

The new antibiotics may be obtained from the appropriate precursor antibiotic, which is identified by the physical parameters given in the aforementioned specification, by recrystallization from a suitable organic solvent. Examples of such solvents include alcohols, especially aliphatic alcohols, such as isopropyl alcohol and halogenated hydrocarbons, especially chlorinated hydrocarbons such as methylene chloride and chloroform.

The new antibiotics when derived from the precursor antibiotic in its free form may be transformed into O-acyl or ether derivatives or salts with bases. Examples of such derivatives include the methyl ether of R-451D(X) and R-451D(X)-acetate.

The precursor antibiotics which, on recrystallization, provide the new antibiotics with an enhanced utility, may be obtained following the procedures described in the aforementioned specificati n. Thus, R-451B may be obtained by the fermentation of M. carbonacea employing the procedure of Example 4 (parts A—E) followed by its separation from the crude antibiotic substance using the two-stage partition chromatographic procedure described in Example 8 (parts A and B). An alternative procedure for the separation of R-451B from the crude antibiotic substance of Example 4 involves partition chromatography on Florisil (" Florisil " which is an activated magnesium silicate is a Registered Trade Mark) as follows:

Prepare a bed of 22.5" height and 1.75" diam. by pouring a methylene chloride slurry of 500 grams of Florisil 60—100 mesh, activated for 18 hours at 105° C, into a glass column. The hold-up volume of the bed is 1100 ml. Dissolve 50 g. of crude antibiotic amorphous precipitate (obtained in Example 4) in 300 ml. of methylene chloride and add the clear solution to the Florisil column. Elute by passing four hold-up volumes of methylene chloride, followed by eight hold-up volumes of methylene chloride containing 5% acetone, eight hold-up volumes of methylene chloride containing 10% acetone, 8-hold-up volumes of methylene chloride containing 20% acetone, 8 hold-up volumes of methylene chloride containing 50% acetone, and finally 16 hold-up volumes of pure acetone. The results of such a procedure are set forth in the following Table I:

TABLE I
Florisil Chromatography of Crude Antibiotics

Eluant	Eluate (ml.)	Weight of Residue	Paper bioautographic pattern; R_F
CH_2Cl_2	4400	10.2 g.	0.43(D); 0.56(E)
„ +5% acetone	8800	8.1 g.	0.45(D)
„ +10% acetone	8800	13.2 g.	0.43(D)
„ +20% acetone	8800	2.3 g.	0.45(D); 0.24(B)
„ +50% acetone	8800	6.3 g.	0.23(B)
100% acetone	8800	5.4 g.	0.15(?); 0.23(B)

In the foregoing Table, the letter designate in the right-hand column denotes the particular R-451 component present in the particular eluate. From the Table it can b seen that the eluants obtained from the 20%, 50% and 100% acetone eluates, particularly the 50% acet ne eluate, are particularly rich in the antibiotic R-451B.

Similarly, the R-451D antibiotic may be obtained by the procedure of Example 5, in the aforementioned specification.

A suitable, convenient procedure for recrystallizing the precursor antibiotics involves the following:

A quantity of the appropriate precursor antibiotic which may be in free form or in the form of an ether or O-acyl derivative or a salt with a base is dissolved in a minimal quantity of an organic solvent, for instance, an alcohol or a halogenated hydrocarbon, which may be hot so as to facilitate dissolution. Decolourising charcoal, or some other clarifying aid may be added to the solution. The solution is then filtered, and the filtrate cooled in an ice-bath whereupon a precipitate forms. This precipitate is then separated, say by filtration or by centrifuging. It may then be washed, conveniently using a similar solvent to that from which it was recrystallized, but for the washing it should preferably be used cold. The washed R-451B(X) or R-451D(X) in free form, or in the form of a derivative (depending upon whether the precursor antibiotic was used in free form or as the corresponding derivative) may then be dried, for instance, in high vacuum at room temperature. The antibiotics R-451B(X) and R-451D(X) if not already in the form of a derivative may be transformed into an O-acyl derivative or an ether or a salt with a base, using conventional procedures.

The antibiotics according to this invention are usually administered in the form of therapeutic compositions containing in addition to one or more of the antibiotics R-451B(X), R-451D(X) and R-451D(X) acetate, a carrier compatible therewith.

The following examples illustrate the invention.

EXAMPLE 1

The antibiotic R-451B obtained from the crude antibiotic substances resulting from the cultivation of *M. carbonacea* under the conditions outlined in the specification of the aforementioned patent using the separation procedure outlined in Example 8 therein, is purified in the following manner:

The R-451B in the form of an amorphous powder is dissolved in hot isopropyl alcohol. 10% by weight of decolourizing charcoal is added, and the solution stirred for 10 minutes. The solution is then filtered, and the filtrate cooled to 5° C. The precipitate, which is R-451B(X) is collected by filtration, washed with isopropyl alcohol, and dried in high vacuum at room temperature for 24 hours. It has an assay value of 760 units/mg., whereas R-451B has an assay value of 700.

R-451B(X), as produced by the method of this Example, is a colorless substance having the following properties:

I. Melting point: (Koeffler block): 154—157° C.

II. Optical rotation. $[\alpha]_D^{25} = -25.5$ (C=1% in pyridine).

III. Ultraviolet absorption:
η_{max} 288 mμ ($E^{1\%}$=12, in methanol)
η_{max} 296 mμ ($E^{1\%}$=72, in 0.1N methanolic NaOH)

IV. Analysis:

(a) *elemental*	b) *functional groups*
C=51.02%	OCH_3=12.80%
H=6.68%	(C) CH_3=7.15%
N=1.23%	(N) CH_3=2.33%
O=34.72%	
Cl=3.97%	

V. Infrared spectrum: The infra-red spectrum of R-451B(X) when suspended in the hydrocarbon mineral oil sold under the Registered Trade Mark Nujol is reproduced in Figure 1 of the accompanying drawings, wherein λ represents the wavelength in mμ, ν represents the frequency in cm^{-1} and A represents the absorption. The absorption peaks and band characteristics summarized in Table II hereunder, wherein the abbreviations have the following significance: (W=weak, M=moderate, M-S=moderate to strong, S=strong, VS=very strong, sh=shoulder). By way of explanation intended to avoid possible confusion, it is mentioned that the peaks which occur at the following wavelengths (3.5—3.6, 6.78—6.87, 7.24 and 13.85—13.90) are caused by Nujol and consequently they have been omitted from the Table.

TABLE II

λmax (μ)	Peak Strength
2.88	S
3.35—3.48	S(broad)
3.65	W
4.54	W
4.65	W
5.74	S
5.82	sh
6.02	sh
6.10	M
6.35—6.39	M(doublet)
6.45	S
7.30	sh
7.40	sh
7.67—7.80	sh
7.92	sh
7.98	S
8.12	sh
8.30—8.35	S(broad)
8.55	sh
8.75—9.80	S(broad)
10.20—10.26	S(broad)
10.30	sh
10.33	sh
10.61	S
10.85	sh
11.03	M
11.52	M
11.70	sh
11.85—12.00	sh
12.75—12.81	W(broad)
12.98	W
13.54—13.60	M(broad)

VI. Solubility: Soluble in chlorinated hydrocarbons (for example methylene chloride, chloroform), alcohol, acetone, pyridine and dilute alkali. Slightly soluble in benzene; insoluble in ether, hexane and water.

EXAMPLE 2

The starting material of this example is the component R-451D which is separated from the crude R-451 by the procedure outlined in Example 5 of the aforementioned patent. This material, which has an assay value of 1155 units/mg. and a melting point of 138° to 140° C, is converted into R-451D(X) by the following procedure. The R-451D is dissolved in a minimal quantity of hot isopropyl alcohol and to the solution there is added, in an amount equal to 10% of the R-451D, decolourizing charcoal. The solution is filtered and the filtrate cooled in an ice-bath, whereupon a precipitate is formed. This precipitate, which is recovered by filtration, is washed with cold isopropyl alcohol and dried in high vacuum at room temperature. The purified material [R-451D(X)] so-obtained has an assay value of 1450 units/mg. The R-451D(X) is biologically homogeneous when assayed qualitatively against *S. aureus*. When the R-451D is chromatographed on a thin layer of silica gel (Stahl technique) using acetone-benzene (1:1) as the eluting agent followed by sulphuric acid treatment of the developed, dried plate, no other substance but R-451D(X) is detectable.

The R-451D(X) as produced by the procedure of this example is a white amorphous powder having the following physical and chemical properties:

I. Melting point: (Koeffler block) 160—161° C.

II. Analysis:

(a) Quantitative
C=51.79%
H=6.35%
N=1.40%
O=36.35%
Cl=3.98%
OCH_3=13.30%
$C(CH_3)$=6.93%
$N(CH_3)$=1.98%

(b) Qualitative
1. Ninhydrin test—negative
2. Elson-Morgan test—positive
3. Alkaline $KMnO_4$ test—positive
4. Anthrone test—greyish blue
5. 2,4-Dinitrophenylhydrazine test: positive
6. Ehrlich (diphenylamine test): negative
7. Triphenyltetrazolium test: negative

III. Rotation $[\alpha]_D^{25} = -25.3°$ (1% in methanol); $-37.7°$ (1% in pyridine)

IV. Ultraviolet spectrum: λ_{max} at 289 mμ ($E^{1\%}$=22); (In a solution of 6 ml. of 1.0N potassium hydroxide in 100 ml. of methanol, λ_{max} shifts to 295 mμ ($E^{1\%}$=79, methanol)).

V. Infra-red Spectrum: The infrared spectrum of R-451D(X) when suspended in the hydrocarbon mineral oil Nujol (Nujol is a registered trade mark) is reproduced in figure 2 of the accompanying drawings, wherein λ represents the wave length in mμ, γ represents the frequency in cm^{-1}, and A represents the absorption. The absorption peaks and band characteristics are summarized in Table III hereunder, wherein the abbreviations have the following significance: (W=weak, M=moderate, M-S=moderate to strong, S=strong, Sh=shoulder). By way of explanation intended to avoid possible confusion, it is mentioned that the peaks which occur at the following wave lengths (3.7, 6.77—6.82, 7.24 and 13.85—13.90) are caused by mineral oil Nujol and consequently they have been omitted from the table.

TABLE III

λmax (μ)	Peak Strength
2.89	S
3.35—3.48	S(broad)
5.73	S
6.12	W
6.35	sh
6.45	S
7.10	sh
7.30	sh
7.42	S
7.68—7.84	S(broad)
7.99	S
8.33	S
8.48—8.70	sh
8.84—9.17	S(broad)
9.57	S(broad)
10.00	sh
10.22—10.26	S(doublet)
10.55	S
10.91	sh
11.00	M
11.50—11.60	M(broad)
11.66	sh
11.90—12.00	M(broad)
12.25—12.35	W(broad)
12.70—12.85	W(broad)
13.00	W
13.52—13.60	W(broad)
14.42—14.58	W(broad)

VI. *Solubility*:
Very soluble in:
chloroform, methylene chloride, acetone and methanol and 0.1N sodium hydroxide
Sparingly soluble in ether.
Insoluble in:
petroleum ether, toluene, benzene, water, 10% sodium bicarbonate (aqueous) and 10% sodium carbonate (aqueous)

VII. *Stability*:
R-451D(X) is stable at 0° C. and in the dark. In methanol solvent containing a trace of pyridine, R-451D is stable for at least two weeks.
Rapid deactivation occurs at a pH below 5.5 whereas at pH above 7 and up to 12 the activity is retained for a few days.
Upon treatment with 0.1N methanolic HCl at room temperature for 16 hours the antibiotic activity is destroyed. Treatment of the acidic mixture with a stoichiometric amount of sodium bicarbonate (2% aqueous solution), followed by removal of the methanol by concentration *in vacuo* and extraction with chloroform yields a mixture of five components as determined by thin layer chromatography on silica gel using benzene acetone (75:25) as a solvent and sulfuric acid as a reagent spray. All components are less polar than R-451D(X).
Upon treatment with 1.0N NaOH at room temperature for 16 hours the activity of R-451D(X) is completely retained.

The R-451D(X) may be converted into any one of a number of derivatives by reaction with the appropriate reagent. The two examples which follow illustrate the preparation of two typical derivatives of R-451D(X) and the properties of one of them.

EXAMPLE 3

Dissolve 25 mg. R-451D(X) in 1 ml. of pyridine. Add 0.4 ml. acetic anhydride and allow the mixture to stand overnight. Filter the precipitate, wash with water to remove pyridine and acetic acid, dry over phosphorous pentoxide in high vacuum overnight. Yield: 11 mg. colourless amorphous powder, m.p. 135—136° C. Isolate a second crop of material from the mother liquor of above precipitate by cooling the solution overnight in a refrigerator (5° C), filtering the precipitate washing it free from any pyridine and acetic anhydride and drying it as above. Yield: 6 mg. of a colourless amorphous powder. Purify by dissolving the crude acetylation product in methylene chloride and adding thereto decolourizing charcoal. Filter and add hexane to the clear filtrate. Filter the precipitate and air dry.

When chromatographed on thin layer, using a silica gel, (Silica Gel G. E. Merck A.G., Darmstadt, West Germany) as adsorbent and acetone:benzene (1:1) as eluting system and upon spraying of the developed and dried plate with sulphuric acid there is obtained a single component of R_F value 0.87. Upon alkaline hydrolysis in methanol, R-451D(X) is regenerated.

The R-451D(X) acetate obtained by the procedure of this example has the following properties:

I Melting point: 151—160° C

II Empirical analysis: C=53.08%, H=7.08%, N=0.77%

III Ultra-violet spectrum: the ultra-violet absorption maximum is a 286 mμ ($E^{1\%}=9.20$)

IV Infra-red spectrum: the absorption bands in the infrared spectrum in Nujol are at 2.85, 5.57, 5.71, 6.14, 6.45, 8.02, 8.38, 8.88, 9.17, 9.55 μ

V Optical rotation: $[\alpha]_{1,}^{25}=-25.4°$ (1% in methanol)

EXAMPLE 4

Dissolve 500 mg. R-451D(X) in 11 ml. of ethyl acetate and 11 ml. of ethyl ether. Add an excess of an ethereal solution of diazomethane and let the yellow solution stand at 5° C for 72 hours. Evaporate the solution to dryness under a stream of nitrogen. Dissolve the crude product in 5 ml. of isopropyl alcohol, refrigerate and collect the precipitate by filtration. Wash with cold isopropyl alcohol and dry obtaining 260 mg. of the methyl ether as colourless prisms, m.p. 161—162° C; $[\alpha]_D^{25}=-17.7°$ (1% in pyridine); λ_{max} 288 mμ ($E^{1\%}=13.7$).

BIOLOGICAL PROPERTIES OF THE ANTIBIOTICS R-451B(X) AND R-451D(X)

The antibiotics R-451B(X) and R-451D(X) exhibit a broad range of anti-microbial activity against gram-positive pathogenic microorganisms. R-451B(X) and R-451D(X) are of particular value in combatting infections produced by penicillin-resistant microorganisms such as certain strains of *Straphylococcus aureus* and are of value in combatting certain microorganisms which are susceptible to destruction by the penicillins. It is known that many disease manifestations are caused by gram-positive organisms (such as *Streptococcus, Staphylococcus, Pneumococcus,* and the like). These are properly controlled and

treated by means of the antibiotics R-451B(X) and R-451D(X). A particular manifestation is infectious bovine-mastitis which is generally caused by species of *Staphylococcus* (*aureus*) and *Streptococcus* (*agalactiae, dysgalactiae,* and *uberis*). These antibiotics effect essentially complete cure of the disease after a relatively brief regimen of administration. Further high potency and effect has been elicited against pathogenic avian strains of pleuropneumonia-like organisms and accordingly these antibiotics are of value to chicken breeders and egg farmers.

The antibiotics by virtue of their antibacterial action against gram-positive microorganisms such as *Staphylococcus aureus* and *Bacillus subtilis,* for example, are advantageously employed as laboratory reagents when attempting to determine the presence of gram-negative organisms. They may be used to inhibit overgrowth of such organisms in culture media, either alone or in combination with other antibacterial agents to reduce or eliminate the heavy overgrowth of gram-positive organisms permitting the determination of gram-negative organisms such as *Klebsiella pneumoniae* or *Escherichia coli* in cultures obtained in diagnostic procedures. As such reagents they may be employed in solutions such as in alcohol.

In view of their action against gram-positive organisms, the antibiotics described herein may be used to "sterilize" equipment such as in operating rooms and hospital wards.

The comparative *in vitro* activities of R-415B(X) (as produced by Example 1) and R-451D(X) (as produced by Example 2) are set forth in Table IV which follows. The susceptibility of the test microorganism to the antibiotic was determined by standard tube dilution methods. In each instance, 10^{-5} dilutions of 24 hour broth cultures were employed as inoculum with the end points taken after incubation for 24 hours at 37° C. in a Difco Pen-assay broth medium (Difco is a Registered Trade Mark) (Difco Labs., Detroit, Michigan). In the table, the activity of the antibiotic is expressed in units per milliliter, a unit being that amount of test substance required to produce a 15 mm zone of inhibition with a steel cylinder of 6.5 mm outside diameter.

TABLE IV

Antibiotic Spectrum of
R-451B(X) and R-451D(X)
(in vitro)

Test Microorganism		Minimal Inhibiting Concentration (units/ml.)	
		R-451B(X)	R-451D(X)
Bacillus cereus	QMCC B964	0.3	0.025
Bacillus megatherium	ATCC 5773	1.2	0.15
Bacillus subtilis	ATCC 6633	1.2	0.15
Sarcina lutea	ATCC 9341	1.2	0.3
Staphylococcus aureus	ATCC 6538	0.15	0.025
" "	ATCC 6538P	0.15	0.025
" "	ATCC 12715	0.6	0.15
" "	ATCC 9996	0.15	0.075
" "	ATCC 1163	0.6	0.3
" "	Gray	0.3	0.025
" "	Smith	0.6	0.15
" "	DA 2027-[1]—2036	—	each 0.25
(10 Clinical isolates resistant to penicillin, streptomycin, tetracycline and erythromycin)			
Diplococcus pneumoniae[2] DA 150		—	0.25
Streptococcus pyogenes[2] DA 21		0.15	0.0075
Streptococcus hemolyticus		0.1	0.01
Streptocossus faecalis	ATCC 10541	0.3	0.025

[1] DA refers to strain identity in private collection of Schering Corporation, Bloomfield, New Jersey.
[2] Brain-heart infusion broth + 0.5% of human serum.

The *in vivo* activity of R-451B(X) and R-451D(X) has been elicited pharmacologically in a standard test animal (mouse 18—20 g.) against certain pathogenic microorganisms. The standard test procedure employed is as follows: Thirty mice were infected with an inoculum of the particular pathogen administered by intraperitoneal injection. Twenty mice were then treated by subcutaneous injection of the antibiotic dissolved in ethanol (2 parts), Tween 80 (0.5 part) (Tween is a Registered Trade Mark), peanut oil (8.5 parts) and the injection administered in two equally divided daily dosages. Ten mice were maintained as controls, that is, untreated. All control animals died within 18 hours. The protecting dose relative to survival of 50% of the treated animals (PD/50) for 48 hours was evaluated to be as follows in conjunction with the particular pathogen.

TABLE V

In Vivo Activity of R-451B(X) and R-451D(X)

Pathogen	PD/50 (mg./kg.)	
	R-451B(X)	R-451D(X)
Streptococcus pyogenes	L 1.3	3.75
Staphylococcus aureus	2.5	12.5
Diplococcus pneumoniae	—	3.75

The acute toxicity of these antibiotics in the standard test animal (mouse 18—20 g.) is as follows:

TABLE VI

Acute Toxicity in Mice of R-451B(X) and R-451D(X)

Mode of Administration	LD/50 (mg./kg.)	
	R-451B(X)	R-451D(X)
Subcutaneous	2500	1000
Intraperitoneal	750	500

WHAT WE CLAIM IS:—

1. An antibiotic identified as R-451B(X) which has a melting point (as determined on the Koeffler block) of 154—157° C, an elementary analysis C=51.02%, H=6.68%, N=1.23%, O=34.72%, Cl=3.97%, an optical rotation $[\alpha]_D^{25} = -25.5$ (1% in pyridine), an ultra-violet absorption maximum at 288 mμ with $E^{1\%}$ in methanol equal to about 12 and an infra-red spectrum when suspended in the hydrocarbon oil Nujol substantially as shown in Figure 1 herein.

2. An antibiotic identified as R-451D(X) which has a melting point (as determined on the Koeffler block) of 160—161° C, an elementary analysis C=51.79%, H=6.35%, N=1.40%, O=36.35%, Cl=3.98%, an optical rotation $[\alpha]_D^{25} = -25.3°$ (1% in methanol), an ultra-violet absorption maximum at 289 mμ with $E^{1\%}$ in methanol equal to about 22, and an infra-red spectrum when suspended in the hydrocarbon oil Nujol substantially as shown in Figure 2 herein.

3. R-451D(X) acetate.

4. Therapeutic compositions containing one or more of the antibiotics identified as R-451B(X), R-451D(X) and R-451D(X) acetate in association with a carrier.

5. Process for the production of an antibiotic identified as R-451B(X) or R-451D(X) or an ether or O-acyl derivative thereof or a salt thereof with a base which comprises recrystallizing from an organic solvent an antibiotic identified as R-451B or R-451D or a corresponding derivative or salt thereof.

6. Process as claimed in claim 5, in which R-451B(X) or R-451D(X) is produced, followed by conversion of the R-451B(X) or R-451D(X) to an ether or O-acyl derivative thereof or a salt thereof with a base.

7. Process as claimed in claim 5, in which the organic solvent is an aliphatic alcohol or a halogenated hydrocarbon.

8. Process as claimed in claim 7, in which the aliphatic alcohol is isopropyl alcohol.

9. Process as claimed in claim 7, in which the halogenated hydrocarbon is methylene chloride.

10. Process as claimed in any one of claims 5 or 7 to 9, in which the antibiotic is dissolved in a minimal quantity of an organic solvent, a clarifying aid such as decolourizing

charcoal is added, the solution filtered and the filtrate cooled, whereupon a precipitate forms.

11. Process as claimed in claim 10, which includes the further steps of washing the precipitate and then drying it.

12. Process for the production of the antibiotics identified as R-451B(X) or R-451D(X) or ether or O-acyl derivatives thereof or salts thereof with bases substantially as described and as exemplified herein.

13. R-451B(X) and R-451D(X) and ether and O-acyl derivatives thereof and salts thereof with bases whenever obtained by the process claimed in any one of claims 5 to 12.

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1,074,235 COMPLETE SPECIFICATION
1 SHEET

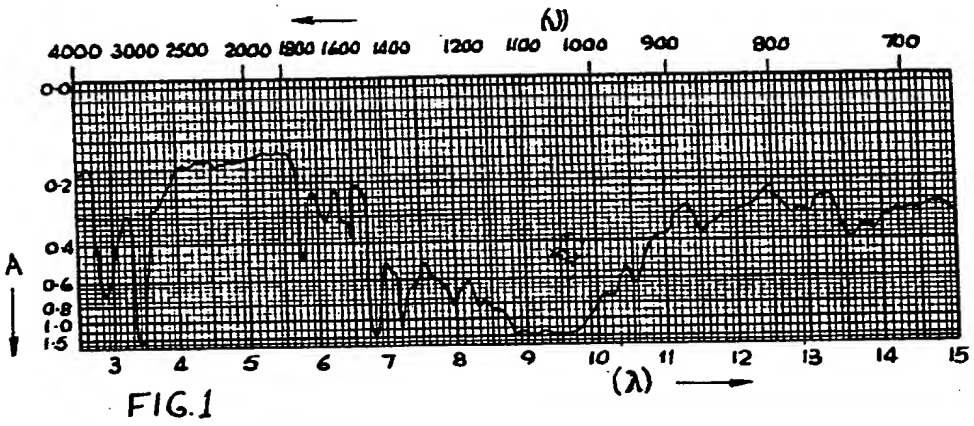

FIG. 1

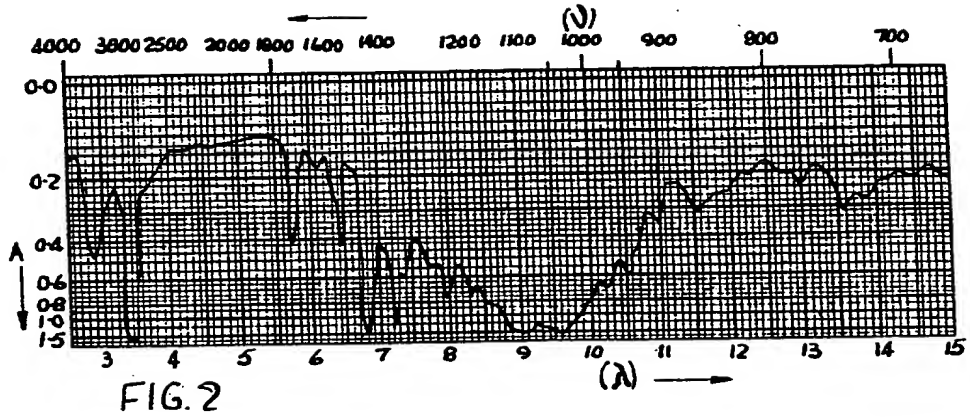

FIG. 2